The oncogenic potential of autocrine human growth hormone in breast cancer

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Given the mortality associated with metastatic breast cancer, the identity and role of factors promoting this process are of major health significance, and the study by Mukhina *et al.* (1) in this issue of PNAS provides such a factor. The important role of estrogens in breast cancer is exemplified by the clinical value of antiestrogens and aromatase inhibitors and by the utility of estrogen receptor status in determining patient prognosis. Increased *Her2/Neu* expression in estrogen receptor-negative patients is an indicator of poor prognosis associated with metastasis, and it has resulted in the development of a clinically useful monoclonal antibody directed to the extracellular domain of HER2, Trastuzumab (Herceptin). HER2 status also provides a useful indicator of the response of breast cancer to other therapies such as topoisomerase inhibitors and anthracyclines. The paralog of the HER2 ligand, heregulin, epidermal growth factor, is an important mammary gland developmental factor, as is estrogen. One might therefore predict that other key hormones and growth factors regulating mammary gland development would be candidate promoters for breast cancer. For example, growth hormone (GH) has important roles in ductal elongation and the differentiation of ductal epithelia into terminal end buds, whereas prolactin is necessary for normal lobular epithelial cell proliferation and secretory function. Attention has focused on prolactin as a potential breast tumor promoter because it was early shown to act in this way with rat mammary carcinomas (2), and overexpression of prolactin in mammary tissue induces tumors in mice (3). The situation in human breast cancer is not so clear, because both prolactin and GH are able to activate the prolactin receptor in humans, and the homologous GH and prolactin receptors activate very similar signaling pathways. These class 1 cytokine receptors activate not only the Janus kinase 2 and signal transducers and activators of transcription 5 (JAK2/STAT5) and JAK2/STAT3 pathways, but also Src family kinases, leading to phospholipase C γ, extracellular signal-regulated kinase (ERK), and phosphatidylinositol 3-kinase (PI 3-kinase) pathway activation. These pathways are capable of increasing cell proliferation, survival, and motility, and ERK activation has been implicated in
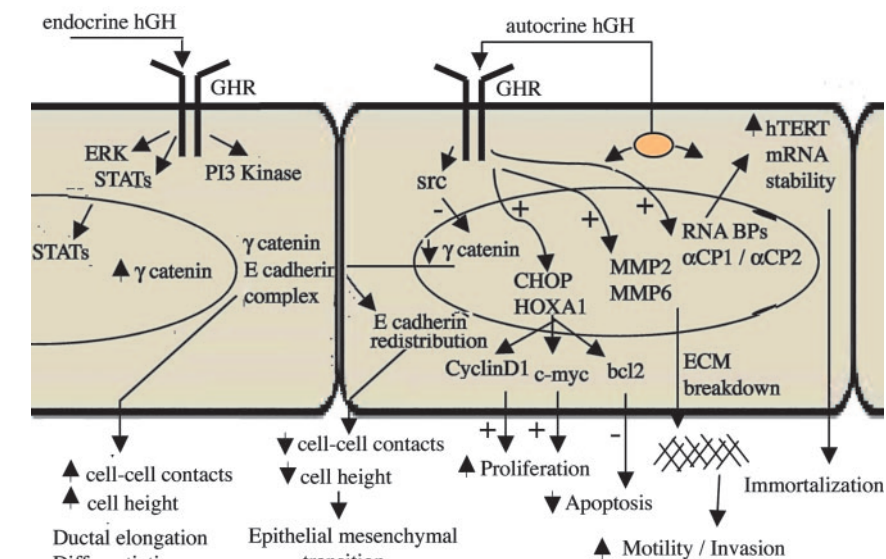

Fig. 1. Endocrine hGH versus autocrine hGH actions on mammary epithelial cells. GHR, GH receptor; BPs, binding proteins; ECM, extracellular matrix.

metastasis. However, there is a relatively poor correlation between circulating prolactin and GH levels in breast cancer incidence and progression, and the data supporting an increased incidence of breast cancer in acromegaly are controversial (4). The latter increase, together with the elevated colon cancer and thyroid nodule risk in acromegaly, has been viewed as resulting from increased free plasma insulin-like growth factor 1 (IGF-1), IGF-1 being the mediator of the somatotrophic actions of GH. Indeed, there is good correlative evidence in large populations for a relationship between free IGF-1 and breast cancer risk (4). The finding that pituitary-GH-deficient dwarf rats with low circulating IGF-1 are resistant to carcinogen-induced mammary cancer supports this view (5).

These considerations have been to an extent confounded by the demonstration that both GH and prolactin can be synthesized within the mammary gland tissue, and therefore are candidates for autocrine/paracrine actions independent of plasma IGF-1. GH is known to be expressed in a number of nonpituitary sites, including lymphocytes, placenta, and brain (6), but GH expression in mammary tissue was initially shown in dogs in response to progesterone administration, where it is produced in sufficient quantity to produce acromegalic symptoms (7). Later studies have extended this observation to other species, including humans (7). More recently, Raccurt *et al.* (8) have shown that in human breast tissue, increased human GH (hGH) expression is associated with increased epithelial cell proliferation, and that metastatic mammary carcinoma cells have the highest levels of hGH expression. It may also be significant that vitamin D, a known inhibitor of mammary carcinogenesis, inhibits hGH transcript expression in human mammary carcinoma cells (9).

The study by Mukhina *et al.* (1) provides a clear demonstration that autocrine production of hGH confers an invasive phenotype on mammary carcinoma cells and that this is the result of an epithelial–mesenchymal transition. As shown in Fig. 1, this phenotypic conversion is associated with a loss of expression of plakoglobin, (γ-catenin), together with a relocalization of E-cadherin to the cytoplasm. Notably, an increased secretion of matrix metalloproteases (MMPs) 2 and 9 is also evident, along with increased cell migration and invasion through Matrigel-coated filters. Migration

and invasion were both blocked by specific src-kinase inhibitors. The *in vitro* evidence for invasive phenotype is supported by studies in immunocompromised mice showing diffuse infiltration of autocrine GH-secreting MCF-7 breast cancer cells into mammary gland stroma, together with islands of tumor cells removed from the main tumor mass.

The mechanistic basis for these observations is not yet fully defined, but there are other studies from Lobie's group that shed light on the subject. First, autocrine hGH up-regulates the expression of *HOXA1*, a potent mammary oncogene that is required for increased c-myc, cyclin D1, and Bcl-2 expression (10). Second, autocrine GH up-regulates gadd153 (CHOP), which was found to provide enhanced protection from apoptosis (11), and down-regulates expression of transcripts for p53-regulated placental transforming growth factor-β (PTGF-β). The latter is known to inhibit GH-stimulated cyclin D1 expression and to promote apoptosis (12). Most recently, this group reported at the International Congress of Endocrinology[†] that autocrine hGH increases telomerase catalytic subunit (hTERT) transcript levels by stabilizing the message, and increased TERT is known to immortalize human mammary epithelial cells. The means used by the activated GH receptor to elicit these changes in gene expression could include activation of the JAK2/STAT3 pathway (13), activation of src kinases, and transactivation of the ErbB-2 receptor (14). The latter two would lead to ERK activation, which is known to promote mammary carcinogenesis (15). Indeed, inhibition of this pathway with mitogen-activated protein kinase kinase (MEK) inhibitor abrogates the increased proliferation seen in autocrine hGH expressing cells (16). Taken together, these studies provide a mechanistic base for the observed onco-

genic transformation resulting from autocrine GH production (Fig. 1).

A key unresolved question is why autocrine GH is so much more effective than exogenous GH in promoting the transformed phenotype *in vitro*. Concordant with this increased effectiveness, the high levels of circulating hGH present in acromegaly are not associated with a large increase in breast cancer risk, and there is no evidence for increased breast cancer progression in GH–replaced individuals. A reasonable hypothesis is that, as well as acting at the cell surface, GH can also act within the cell to promote the transformed phenotype, perhaps within the endoplasmic reticulum and Golgi. The resulting signal may be insufficient to generate negative regulators such as the suppressors of cytokine signaling, but because it is sustained, is sufficient to promote cell proliferation and transformation. Alternatively, GH receptor is transported to the nucleus in response to GH stimulation, and ≈50% of cellular JAK2 is nuclear (17). Autocrine GH could thus act within the nucleus to regulate expression of the key transforming genes described above. Indeed, we have shown that forced translocation of the GH receptor to the nucleus is sufficient to induce the transformed phenotype in pro-B cells, resulting in metastasizing tumors in severe combined immunodeficient (SCID) mice (B.C.-C., G. M. Boyle, P. G. Parsons, and M.J.W., unpublished data). It is also possible that GH acts in a novel manner within the cell, for example, consequent to its reported peptidylglycine monooxygenase activity (18).

What potential therapeutic approaches stem from the study Mukhina *et al.* (1)? Given the autocrine origin of the hGH, inhibition of pituitary GH secretion is unlikely to be effective. Fortunately, there is an effective clinical GH antagonist currently in use for acromegaly, Trovert or B2036. This mechanism-

based inhibitor of GH signaling works by binding to the receptor and blocking the conformational change that results in GH receptor activation. That the Gly-120 → Arg substitution creates a GH antagonist was discovered in John Kopchick's laboratory, but the low affinity of this mutant required affinity maturation by phage display to make an effective drug, which was undertaken in Jim Wells' laboratory at Genentech (19). The eight resulting substitutions also restricted the specificity of this antagonist to hGH receptors, allowing Kaulsay *et al.* (20) to show that blockade of GH and not prolactin receptor was able to inhibit the proliferative and cell motility activity of autocrine hGH in MCF-7 mammary carcinoma cells. This result provides a clear avenue to therapeutic trials in breast cancer, a view that is supported by the widespread extent of GH receptor expression in breast cancer (21). This therapy would have the additional advantage of lowering plasma IGF-1 level, which itself would be predicted to slow tumor progression. However, it may not be the complete drug, because there is a good deal of evidence that autocrine prolactin is a tumor promoter in breast cancer, and B2036 would not target the prolactin receptor. Moreover, prolactin receptor expression is present in ≈70% of breast biopsies and is reportedly increased in breast cancer (21, 22), whereas expression of the shorter, dominant-negative, form of this receptor is decreased (23). Prolactin itself is produced by 70% of breast cancer lines (24, 25) and by a similar proportion of biopsies, and antibody neutralization of secreted autocrine prolactin decreases the proliferation of breast cancer lines (25). These two paralogous cytokine hormones thus have parallel and interconnected roles in breast cancer as they do in breast development. Targeting of both simultaneously with a broader spectrum version of B2036 is likely to offer the most effective therapy in breast cancer.

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